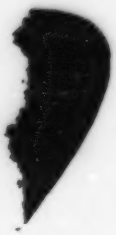

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Immunochromatographic assays for identification of biological agents: NATO SIBCA Exercise I

R. Elaine Fulton
Defence Research Establishment Suffield

Laurie L. Stadnyk
Defence Research Establishment Suffield

Technical Report
DRES TR 2000-154
September 2000

National Defence Défense nationale

Canada

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R. Elaine Fulton and Laurie L. Stadnyk
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Abstract

In March 1999, the NATO Panel VII Subgroup on Sampling and Identification of Biological and Chemical Agents (SIBCA) conducted the first international training exercise on identification of biological agents. Eleven NATO national laboratories participated in the exercise: Canada, France, Germany (two laboratories), Hungary, Italy, Netherlands, Norway, Poland, United Kingdom, and United States. Participating laboratories were sent five sample unknowns, four of which contained biological agents and one blank containing buffer only. Participants were advised that biological agents could consist of any one of the following 10 gamma-irradiated organisms: *Bacillus anthracis*, *Yersinia pestis*, *Vibrio cholerae*, Venezuelan Equine Encephalitis (VEE) virus, *Francisella tularensis*, *Brucella melitensis*, *Burkholderia mallei*, Yellow Fever virus, Vaccinia virus, or *Coxiella burnetii*. The participating laboratory for Canada was the Defence Research Establishment Suffield (DRES). For the training exercise, DRES screened sample unknowns by two different antibody-based identification technologies, one of which was the immunochromatographic assay (ICA). ICAs for six of the 10 possible biological agents were available from one or both of two commercial sources and SIBCA samples were screened for these six agents. Cross-reactivity of some ICAs with heterologous agents, as well as non-reactivity of some positive agent control materials on some ICAs, were observed. However, comparison of ICA results with the known identity of agents in SIBCA sample unknowns indicated 100% correct identification of agents for which ICAs were available and validated.

Résumé

Au mois de mars 1999, le Sous-groupe sur le prélèvement d'échantillons et l'identification des agents chimiques et biologiques (SIBCA) du Groupe VII de l'OTAN a mené le premier exercice d'entraînement international sur l'identification des agents biologiques. Onze laboratoires nationaux de l'OTAN y ont participé : ceux du Canada, de la France, de l'Allemagne (deux laboratoires), de la Hongrie, de l'Italie, des Pays-Bas, de la Norvège, de la Pologne, du Royaume-Uni et des États-Unis. On a fait parvenir aux laboratoires participants cinq échantillons inconnus, dont quatre contenaient des agents biologiques et l'un ne contenait que des tampons. On a informé les participants que les échantillons pouvaient contenir n'importe lequel de ces dix organismes irradiés aux rayons gamma : *Bacillus anthracis*, *Yersinia pestis*, *Brucella melitensis*, *Francisella tularensis*, *Vibrio cholerae*, *Burkholderia mallei*, le virus de l'encéphalite équine du Venezuela (EEV), le virus de la vaccine, *Coxiella burnetii* ou le virus de la fièvre jaune. Le laboratoire canadien participant était le Centre de recherches pour la défense Suffield (CRDS). Pour cet exercice, le CRDS a analysé des échantillons inconnus au moyen de deux techniques d'identification différentes basées sur les anticorps, dont l'une était le test immunochromatographique (TI). Il était possible de se procurer les TI pour six des dix agents biologiques auprès d'une ou de deux sources commerciales, et on a cherché à détecter ces six agents dans les échantillons du SIBCA. La réactivité croisée de certains TI avec des agents hétérologues, de même que la non-réactivité de certains échantillons de contrôle positifs dans certains TI ont pu être observées. Cependant, la comparaison des résultats des TI à l'identité connue des agents contenus dans les échantillons inconnus du SIBCA a révélé que l'identification des agents pour lesquels des TI étaient disponibles et validés était correcte à 100 %.

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Author

R. Elaine Fulton and Laurie L. Stadnyk

Approved by

Dr. C. Boulet

Head/Chemical and Biological Defence Section

Approved for release by

Dr. R. Herring

Chair DRP/DRES

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Executive summary

Introduction

NATO Forces may be required to carry out military or peace keeping operations in areas of the world where there is a threat of attack with biological agents or where the occurrence of biological attack is suspected or confirmed. Under such circumstances, NATO Forces would be expected to take samples of materials suspected of containing biological agents and to forward same to respective national laboratories, where procedures would be carried out to identify agent unknowns. In order to assess national capabilities in the NATO laboratories for identification of biological agents in samples, the NATO Panel VII Subgroup on Sampling and Identification of Biological and Chemical Agents (SIBCA) organized international training exercises in which participating nations were requested to identify, within a given time period, agents in sample unknowns.

The first SIBCA training exercise for biological agents i.e., SIBCA exercise I, was held in March 1999. Eleven national laboratories participated in the exercise: Canada, France, Germany (two laboratories), Hungary, Italy, Netherlands, Norway, Poland, United Kingdom, and United States. Participant nations were advised that biological agents could consist of any one of the following 10 gamma-irradiated organisms: *Bacillus anthracis*, *Yersinia pestis*, *Vibrio cholerae*, Venezuelan Equine Encephalitis (VEE) virus, *Francisella tularensis*, *Brucella melitensis*, *Burkholderia mallei*, Yellow Fever virus, Vaccinia virus, or *Coxiella burnetii*. The participating laboratory for Canada was the Defence Research Establishment Suffield (DRES). DRES screened sample unknowns by two different antibody-based identification technologies, one of which was the immunochromatographic assay (ICA). This report describes the evaluation and validation of ICAs available from two different commercial sources and the results obtained when these ICAs were used to screen the SIBCA exercise I samples for agent unknowns.

Results

ICAs were purchased or gifted from New Horizons Diagnostics Corp. (NHDC) and/or Majesco Biologicals Inc. (MBI). ICAs were available from one or both manufacturers for Vaccinia virus, *Yersinia pestis*, *Francisella tularensis*, *Brucella*, *Bacillus anthracis*, and *Vibrio cholerae*. ICAs were unattainable for *Burkholderia mallei*, VEE virus, Yellow fever virus, and *Coxiella burnetii*. Concurrent to screening of SIBCA exercise samples, agent-specific ICAs were evaluated for sensitivity in detection of the homologous agent and specificity against a panel of heterologous agents. Comparison of results obtained by ICA with the known content of agents in exercise samples indicated that, for those organisms for which ICAs were available and validated for use in the exercise, there was 100% correct identification of agents and a 0% false negative identification rate. However, results also indicated that there were extensive heterologous agent cross-reactivities in ICAs from one commercial source and lack of reactivity with formalin-inactivated homologous agents (positive control samples) from another commercial source.

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Significance of results

ICAs are installed as the first generation identification technology on the Canadian Integrated Biological Agent Detection System (CIBADS). Hence, Canada needs to identify a consistent source of supply of high quality ICAs to satisfy the continuing requirements of CIBADS. SIBCA exercise I provided DRES with an excellent opportunity to assess the availability of ICAs for the agents of military relevance and to evaluate their quality and likely utility as immunodiagnostic tools. The results described in this report reflect the state-of-the-art in development of ICAs at the time of SIBCA exercise I. Issues relating to availability, ease of handling, cross-reactivity with heterologous agents, and non-reactivity with homologous agents were encountered as obstacles in assessment of the ICAs available for this exercise. However, in spite of these problems, results indicated that, for those agent-specific ICAs that were available and validated for use in the exercise, there was 100% correct identification of agent sample unknowns, thus demonstrating the potential utility of ICAs as immunodiagnostic tools for identification of biological agents of military relevance. In addition, an important observation was made, during the course of this exercise, relating to differences in assay reactivity associated with the nature of antigen used to induce the detecting antibodies. In this regard, it was observed that live agents reacted well in ICAs that incorporated antibodies raised against gamma-irradiated antigens, while formalin-inactivated agents were non-reactive in the same agent-specific ICAs. This observation suggests that the method of inactivation of agents used as immunogens in the induction of antibodies is an important parameter affecting the immunoreactivity and, hence, biodetection capability of the antibodies produced.

Future goals

DRES will continue to assess "off-the-shelf" ICAs as they become available from various commercial sources for identification of agents of concern to the Canadian Forces (CF). In addition, DRES will continue to evaluate available ICAs as immunodiagnostic tools for identification of agents in sample unknowns, by participation in future SIBCA exercises, if and when these should occur. Furthermore, DRES is developing expertise domestically, in Canada, for the development and manufacture of ICAs, through research and development contracts with Canadian industry, and the products developed from these contracts will be evaluated at DRES for bio-identification efficacy.

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Sommaire

Introduction

Les forces de l'OTAN peuvent être appelées à mener des opérations militaires ou de maintien de la paix dans des régions du monde où elles risquent d'être attaquées avec des agents biologiques ou des régions où l'on sait ou soupçonne qu'il y a eu de telles attaques. En pareilles circonstances, on s'attend à ce que les forces de l'OTAN prélèvent des échantillons des substances qu'elles soupçonnent de contenir des agents biologiques et les fassent parvenir à leurs laboratoires nationaux respectifs, où des mesures seront prises pour identifier les agents inconnus. Pour évaluer la capacité des laboratoires nationaux de l'OTAN d'identifier des agents biologiques dans des échantillons, le Sous-groupe sur le prélèvement d'échantillons et l'identification des agents chimiques et biologiques (SIBCA) du Groupe VII de l'OTAN a organisé des exercices d'entraînement internationaux dans le cadre desquels les pays participants ont été appelés à identifier , dans un délai donné, les agents présents dans des échantillons inconnus.

Le premier exercice d'entraînement du SIBCA portant sur les agents biologiques, soit l'exercice I du SIBCA, s'est tenu en mars 1999. Onze laboratoires nationaux de l'OTAN y ont participé : ceux du Canada, de la France, de l'Allemagne (deux laboratoires), de la Hongrie, de l'Italie, des Pays-Bas, de la Norvège, de la Pologne, du Royaume-Uni et des États-Unis. Les pays participants ont été informés du fait que les agents biologiques pouvaient être n'importe lequel de ces dix organismes irradiés aux rayons gamma : *Bacillus anthracis*, *Yersinia pestis*, *Brucella melitensis*, *Francisella tularensis*, *Vibrio cholerae*, *Burkholderia mallei*, le virus de l'encéphalite équine du Venezuela (EEV), le virus de la vaccine, *Coxiella burnetii* ou le virus de la fièvre jaune. Le laboratoire canadien participant était le Centre de recherches pour la défense Suffield (CRDS). Le CRDS a analysé des échantillons inconnus au moyen de deux techniques d'identification différentes basées sur les anticorps, dont l'une était le test immunochromatographique (TI). Ce rapport décrit l'évaluation et la validation des TI pouvant être obtenus de deux sources commerciales différentes et les résultats obtenus lorsque ces TI ont été utilisés pour détecter des agents inconnus dans les échantillons de l'exercice I du SIBCA.

Résultats

Les TI ont été vendus ou donnés par New Horizons Diagnostics Corp. (NHDC) et/ou Majesco Biologicals Inc. (MBI). Les TI pouvaient être obtenus de l'un ou des deux fabricants pour le virus de la vaccine, *Yersinia pestis*, *Francisella tularensis*, *Brucella*, *Bacillus anthracis* et *Vibrio cholerae*. Ils ne pouvaient être obtenus pour *Burkholderia mallei*, le virus de l'encéphalite équine du Venezuela (EEV), le virus de la fièvre jaune et *Coxiella burnetii*. Parallèlement à l'analyse des échantillons de l'exercice du SIBCA, on a évalué les TI spécifiques des agents en vue de vérifier leur sensibilité dans la détection de l'agent homologue et leur spécificité par rapport à un panel d'agents hétérologues. La comparaison des résultats obtenus par les TI au contenu connu des échantillons de l'exercice a révélé que, dans le cas des organismes pour lesquels des TI étaient disponibles et validés aux fins de l'exercice, l'identification des agents était correcte à 100 % et le taux d'identification de faux

négatifs était de 0 %. Cependant, les résultats indiquaient également que la réactivité croisée avec les agents hétérologues était considérable dans les TI provenant d'une source commerciale et qu'il n'y avait pas de réactivité avec les agents homologues inactivés au formol (échantillons de contrôle positifs) d'une autre source commerciale.

Signification des résultats

Les TI constituent la première génération de technologies d'identification dans le Système canadien intégré de détection des agents biologiques (CIBADS). Le Canada doit donc trouver une source d'approvisionnement régulière de TI de grande qualité pour répondre aux besoins constants du CIBADS. L'exercice I du SIBCA a fourni au CRDS une excellente occasion d'évaluer la disponibilité de TI pour les agents d'usage militaire et d'évaluer leur qualité et leur utilité probable en tant qu'outils d'immunodiagnostic. Les résultats décrits dans ce rapport reflètent les TI de pointe à la date de l'exercice I du SIBCA. Des questions de disponibilité, de facilité de manipulation, de réactivité croisée avec les agents hétérologues et de non-réactivité avec les agents homologues ont constitué des obstacles dans l'évaluation des TI disponibles pour l'exercice. Toutefois, malgré ces problèmes, il est ressorti des résultats que, dans le cas des TI spécifiques d'agents qui étaient disponibles et validés pour l'exercice, l'identification des agents dans les échantillons inconnus était correcte à 100 %, ce qui démontre l'utilité potentielle des TI en tant qu'outils d'immunodiagnostic dans l'identification des agents biologiques d'usage militaire. De plus, une observation importante a été faite au cours de cet exercice relativement aux différences de réactivité des tests associées à la nature de l'antigène utilisé pour induire les anticorps. Il a été observé à cet égard que les agents vivants réagissaient bien dans les TI comportant des anticorps obtenus en réaction à des antigènes irradiés aux rayons gamme, alors que les agents inactivés au formol n'étaient pas réactifs dans les mêmes TI spécifiques d'agents. Cette observation laisse supposer que la méthode d'inactivation des agents utilisés comme immunogènes dans l'induction d'anticorps est un paramètre important qui a un effet sur l'immunoréactivité et, par conséquent, sur la capacité de biodétection des anticorps produits.

Objectifs pour l'avenir

Le CRDS continuera d'évaluer les TI vendus dans le commerce au fur et à mesure qu'ils seront distribués par diverses sources commerciales pour l'identification d'agents que surveillent les Forces canadiennes (FC). De plus, le CRDS développe au Canada même une expertise dans le développement et la fabrication de TI, par le biais de contrats de recherche et développement avec l'industrie canadienne, et il évaluera l'efficacité des produits issus de l'exécution de ces contrats sur le plan de la bio-identification.

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Acknowledgements

The authors wish to thank Jim Hancock, DRES, Canadian Office of Primary Interest on the NATO Panel VII Subgroup on Sampling and Identification of Biological and Chemical Agents (SIBCA), for organizing the Canadian participation in SIBCA exercise I. Thanks are also due to the West Desert Test Center, US Dugway Proving Ground, UT, for provision of the SIBCA exercise I sample unknowns. The assistance of B. Kournikakis, DRES, in procuring, partitioning, and archiving of the SIBCA samples at DRES is also gratefully acknowledged.

Introduction

NATO Forces may be required to carry out military or peace keeping operations in areas of the world where there is a threat of attack with biological agents or where the occurrence of biological attack is suspected or confirmed. Under such circumstances, NATO Forces would be expected to take samples of suspected materials and to forward same to respective national laboratories, where procedures would be undertaken to identify agent unknowns. In order to assess national capabilities in the NATO laboratories for identification of biological agents in samples, the NATO Panel VII Subgroup on Sampling and Identification of Biological and Chemical Agents (SIBCA) organized international training exercises in which participating nations were requested to identify, within a given time period, biological agents in sample unknowns. The results were subsequently referred back to the host nation, for collation and analysis.

The first SIBCA biological agent training exercise i.e., SIBCA exercise I, was held in March 1999. Sample unknowns were provided by the host nation, the West Desert Test Center, United States Dugway Proving Ground (US DPG), Utah. Eleven national laboratories participated in the exercise: Canada, France, Germany (two laboratories), Hungary, Italy, Netherlands, Norway, Poland, United Kingdom, and United States. Each participating laboratory was sent five sample unknowns, four of which contained biological material and one blank containing phosphate buffered saline (PBS) only. Participants were informed that biological material could consist of any of the following 10 cobalt-inactivated agents: *Bacillus anthracis*, *Yersinia pestis*, *Vibrio cholerae*, Venezuelan Equine Encephalitis (VEE) virus, *Francisella tularensis*, *Brucella melitensis*, *Burkholderia mallei*, Yellow Fever virus, Vaccinia virus, or *Coxiella burnetii*. Sample analyses were to be completed within 10 working days.

In Canada, the participating laboratory was the Defence Research Establishment Suffield (DRES), Ralston, AB. DRES assessed samples by two different antibody-based techniques. The primary identification technique was the Threshold® immunoassay (Molecular Devices Corp., Menlo Park, CA), based on Light Addressable Potentiometric Sensor (LAPS) biosensor technology. Results obtained by Threshold® assay were "backed up" or confirmed by immunochromatographic assay (ICA). In addition, samples were investigated by limited application of a genetic-based approach i.e., polymerase chain reaction (PCR). Results of analyses by Threshold® immunoassay and by PCR are reported elsewhere [1, 2, 3]. The report contained herein describes the results obtained on screening of SIBCA exercise I samples by ICA.

ICAs used to screen the SIBCA sample unknowns were purchased from two different commercial sources. Concurrent to screening of SIBCA samples by ICA, agent-specific ICAs were evaluated for sensitivity in detection of the homologous agent and specificity against a panel of heterologous agents. Comparison of results obtained on SIBCA sample unknowns by ICA with the known content of agents in exercise samples, as revealed post-exercise, by the host nation, indicated that, for those organisms for which ICAs were available and validated for use in the exercise, there was 100% correct identification of agents and 0% false negative identification. However, results also indicated there were extensive heterologous agent cross-reactivities in ICAs from one commercial source and lack of reactivity with formalin-inactivated control agents in ICAs from another commercial source. Furthermore, formalin-inactivated agents held in the DRES collection were found, for the most part, to be unsuitable for use in the exercises as positive control reference materials.

Materials and methods

SIBCA test samples

Five SIBCA exercise unknown samples, labelled #110, #137, #143, #157, and #160, were received at DRES. Participants were notified that four of the five samples contained any one of the following cobalt-irradiated agents: *Bacillus anthracis*, *Yersinia pestis*, *Vibrio cholerae*, Venezuelan Equine Encephalitis (VEE) virus, *Francisella tularensis*, *Brucella melitensis*, *Burkholderia mallei*, Yellow Fever virus, Vaccinia virus, or *Coxiella burnetii*. Sample unknowns were suspended in 10 mL PBS and contained either bacteria at a concentration of 10^6 - 10^7 cfu/mL or virus/rickettsia at a concentration of 10^7 - 10^8 pfu/mL; one of the five samples consisted of PBS only. Participants were allowed 10 working days to complete their analyses, after which, results were to be sent to DPG for collation. Samples were stored at DRES at -70 °C prior to use.

Immunochromatographic assays

The following SMART™ ICAs were generous gifts from New Horizons Diagnostics Corp. (NHDC), Columbia, MD: *Brucella melitensis* (Lot 900, Exp 062898); *Francisella tularensis* (Lot 800, Exp 062898); *Yersinia pestis* (Lot 302, Exp 112898); and *Bacillus anthracis* (Lot 503A, Exp 042899 and Lot 504, Exp 122899). *Vibrio cholerae* ICAs (Lot 136, Exp 102899) were purchased from NHDC. All NHDC tickets were received at DRES well in advance of the SIBCA exercises.

The following ICAs were purchased through the agencies of the Canadian Embassy, Washington, DC and the United States Army Joint Program Office for Biological Defense (US JPO-BD), Falls Church, VA, from the manufacturer, Majesco Biologicals Inc. (MBI), Edison, NJ: *Brucella melitensis* (Lot MA8M31, Man Dec 98); *Francisella tularensis* (Lot MU8M21, Man Dec 98); *Yersinia pestis* (Lot ML8M31, Man Jun 98); *Bacillus anthracis* (Lot MN8F11, Man Jun 98); and Vaccinia virus (Lot MS8M21, Man Dec 98). The MBI tickets were received at DRES on the last designated day of the SIBCA exercise.

Homologous and heterologous antigens

ICAs from NHDC and from MBI were evaluated for sensitivity and specificity using the following homologous and heterologous antigens: *Brucella melitensis*: Strain 16M, obtained from Animal Diseases Research Institute (ADRI) (Nepean, ON), formalin-inactivated at DRES; *Francisella tularensis*: Live vaccine strain (LVS), obtained from United States Army Medical Research Institute of Infectious Diseases (USAMRIID), formalin-inactivated at DRES; *Yersinia pestis*: Avirulent strain NCTC 5923 (*Pasteurella pestis*), ATCC #19428 (American Type Culture Collection, Manassas, VA), formalin-inactivated at DRES; *Bacillus anthracis*: Thraxol^R vaccine, Sterne strain, obtained from ADRI, (Lethbridge, AB), formalin-inactivated at DRES; *Vibrio cholerae* 01: formalin-inactivated (provided with kit); and

vaccinia virus vaccine: smallpox live vaccine (Lot 1556-12, Exp Jan 96), purchased from Connaught Laboratories, Willowdale, ON.

In addition, ICAs from MBI were evaluated for reactivity with live strains of *Yersinia pestis*, strain GB; *Brucella melitensis*; and *Bacillus anthracis*, strain Ames. All antigen dilutions were prepared in 10 mM PBS, pH 7.4 (Sigma Chemical Co.).

Assay procedures

Samples for ICA were tested undiluted, in duplicate or singly, on both NHDC and MBI tickets. Because the MBI tickets were not received at DRES until the final day (day 10) of the SIBCA exercise, it was not possible to evaluate these tickets for sensitivity and specificity prior to the exercise. Therefore, sensitivity and specificity evaluation of MBI tickets was performed in parallel with the screening of SIBCA sample unknowns. Similarly, NHDC tickets were assessed for sensitivity and specificity concurrently and in parallel with testing of the SIBCA sample unknowns.

Negative controls used with NHDC ICAs consisted of PBS, Smart™ wash solution (provided with NHDC kits), or NANOpure® water (Barnstead/Thermolyne Corp., Dubuque, IO) (collection fluid for the Canadian Integrated Biological Agent Detector System (CIBADS)). Negative controls used with MBI tickets consisted of PBS or NANOpure® water.

NHDC tickets

Each Smart™ assay kit for detection of bacteria consisted of a bottle of wash solution, plastic disposable pipettes, a test tube holder, and individually wrapped and sealed foil pouches, each containing a test device (ticket), a reaction vial (containing reaction tablet), several cotton swabs, and desiccant. The procedures followed for the assay for *Bacillus anthracis* were those provided by the manufacturer for bacteria (spore) and, briefly, were as follows. Three drops of wash solution and three drops of test sample were added to the reaction vial and the solution was mixed for 3 min, or until the tablet was dissolved. A cotton swab was immersed in the reaction vial solution and the swab then positioned in the upper compartment of the Smart™ device. The device compartment was closed and the lid was pressed firmly closed with the thumb. After 15 min reaction time at room temperature, the lower "results" compartment was opened and the results read. A pink to dark red test spot indicated a positive response. Procedures followed for other agents were basically the same as those described for anthrax, excepting that reaction volumes were measured rather than estimated by drop. Hence, the reaction vial containing the reaction tablet received 100 uL wash solution and 100 uL test sample. With the exception of assays for *Bacillus anthracis*, all test devices had, in addition to a test spot, a negative control spot. Test devices for *Vibrio cholerae* also had a positive control spot.

MBI tickets

ICA tickets from MBI were packaged within clear, plastic "clam-shell" holders that opened and closed through a hinge mechanism and snapped shut through interlocking plastic buttons. Each holder was machined into eight recessed compartments that held eight individual tickets for eight different biological agent tests. The ticket holder was, in turn, sealed, together with desiccant, within a foil pouch. Tickets for the specific agents required for the SIBCA exercise were selected and transferred to a separate holder. The procedures followed for performance of the assays were those provided by the manufacturer and, briefly, were as follows. One hundred uL of test sample was added to the sample well of each ticket. The reaction was allowed to develop for 10 min at room temperature, after which time, the results were read. A positive result was indicated by the presence of two dark red lines, one at the test line location (labelled T) and one at the negative control line location (labelled C). A negative result was indicated by the presence of one line only, located at position C i.e., no reaction at position T.

Results

Five sample unknowns were received at DRES from DPG and analysed by ICA for the presence of biological agents. Samples were screened by tickets obtained from two different commercial sources, as follows: NHDC: *Brucella melitensis*, *Francisella tularensis*, *Yersinia pestis*, *Bacillus anthracis*, and *Vibrio cholerae*; MBI: *Brucella melitensis*, *Francisella tularensis*, *Yersinia pestis*, *Bacillus anthracis*, and Vaccinia virus. No tickets for VEE, *Burkholderia mallei*, yellow fever virus, or *Coxiella burnetii* were available from either manufacturer. Concurrent with screening of SIBCA sample unknowns, ICAs from both manufacturers were evaluated for sensitivity in detection of homologous antigen and specificity with regard to heterologous agents included in the exercise.

NHDC tickets

Sensitivity and specificity

NHDC tickets (*Vibrio cholerae*, *Yersinia pestis*, *Francisella tularensis*, *Brucella melitensis*, and *Bacillus anthracis*) were evaluated for sensitivity and specificity. In addition, tickets were tested for non-specificity in the presence of PBS, Smart™ wash solution, and NANOpure® water, respectively. The results of these evaluations are presented in Table 1. *Vibrio cholerae* tickets reacted strongly with the homologous antigen and did not cross-react with any of the heterologous antigens tested. *Yersinia pestis* tickets gave weak positive reactions when tested with the homologous antigen at $1x10^6$ and $1x10^7$cfu/mL. However, these tickets were also positive, in one of two tickets tested, with *Francisella tularensis* antigen at $1.1x10^8$ cfu/mL. Similarly, one of two tickets tested with *Brucella melitensis* antigen at $1.8x10^8$ cfu/mL was positive. *Francisella tularensis* tickets reacted weakly with *Francisella tularensis* antigen at $1.1x10^7$ cfu/mL but were negative when tested with *Francisella tularensis* antigen at $1.1x10^8$ cfu/mL; *Francisella tularensis* tickets did not, however, exhibit cross-reactivity with any of the other antigens tested. *Brucella melitensis* tickets were negative for all antigens at all concentrations tested. *Bacillus anthracis* tickets cross-reacted with all antigens tested, as well as with PBS and water.

Ticket validation

NHDC tickets were considered valid for the purposes of the training exercise if they were positive with the homologous agent, did not cross-react with the heterologous agents tested, and did not give positive reactions with water or buffer.

NHDC tickets for *Yersinia pestis* cross-reacted with several of the heterologous agents tested; tickets for *Bacillus anthracis* not only cross-reacted with heterologous agents, but also, gave false positive reactions with buffer and water (Table 1). For these reasons, both the *Yersinia pestis* and *Bacillus anthracis* tickets were declared invalid for use in this training exercise. Similarly, *Brucella melitensis* tickets were also invalidated because they did not react with the homologous agent. Only the tickets for *Vibrio cholerae* and *Francisella tularensis* reacted

with the homologous agent and did not cross-react on sensitivity and specificity testing, hence only these tickets were considered reliable and valid for the purposes of the exercise.

SIBCA exercise samples

Results of screening of SIBCA sample unknowns on NHDC tickets are presented in Table 2. None of the SIBCA exercise samples was positive on *Vibrio cholerae*, *Francisella tularensis*, or *Brucella melitensis* tickets. Samples #110, #143, and #157 gave positive reactions on *Yersinia pestis* tickets and all five SIBCA sample unknowns were positive on the *Bacillus anthracis* tickets. Because only the *Vibrio cholerae* and *Franceisella tularensis* NHDC tickets were validated following sensitivity and specificity assessment (see above), results from screening of exercise samples on *Yersinia pestis*, *Bacillus anthracis*, and *Brucella melitensis* tickets were disregarded. None of the SIBCA exercise samples was positive on *Vibrio cholerae* or *Francisella tularensis* tickets, suggesting that these agents were not present in any of the samples.

MBI tickets

Sensitivity and specificity

MBI tickets (Vaccinia virus, *Yersinia pestis*, *Francisella tularensis*, *Brucella melitensis*, and *Bacillus anthracis*) were evaluated for sensitivity and specificity. In addition, these tickets were tested for non-specificity in the presence of PBS and NANOpure® water. The results of these tests are shown in Table 3. Vaccinia tickets reacted well with vaccinia live virus vaccine at 5 doses/mL and did not cross-react with inactivated heterologous agents at any of the dilutions tested. Similarly, *Francisella tularensis* tickets reacted with homologous inactivated agent and did not cross-react with heterologous agents. However, tickets for *Yersinia pestis*, *Brucella melitensis*, and *Bacillus anthracis*, although not cross-reactive with heterologous agents, also did not react with their respective homologous agents, at any of the dilutions tested. As a last resort in validating tickets for *Yersinia pestis*, *Brucella melitensis*, and *Bacillus anthracis* for reactivity with the respective homologous agents, tickets for these agents were taken into the DRES biocontainment level 3 laboratories and tested using unspecified amounts of the respective live agents. Hence, it was observed that the *Yersinia pestis* ticket reacted with live *Yersinia pestis*, the *Brucella melitensis* ticket reacted with live *Brucella melitensis*, and the *Bacillus anthracis* ticket was positive with live *Bacillus anthracis*. The reactivity of *Yersinia pestis*, *Brucella melitensis*, and *Bacillus anthracis* tickets with heterologous live agents was not assessed.

Ticket validation

MBI tickets were considered valid for the purposes of the training exercise if they were positive with the homologous agent, did not cross-react with the heterologous agents tested, and did not give positive reactions with water or buffer.

None of the MBI tickets cross-reacted with any of the killed heterologous agents tested. Similarly, there was no non-specific reactivity with buffer or water. The MBI tickets, however, with the exception of the Vaccinia virus and *Francisella tularensis* tickets, were non-reactive with homologous killed agents but reacted strongly when tested with the

respective live agents. Due to resource limitations i.e., ticket availability, it was not possible to evaluate the MBI tickets for cross-reactivity with live agents. However, the manufacturer gave assurances that extensive cross-reactivity studies had been conducted at the factory and that the tickets were, indeed, agent-specific. Based on these assurances, all MBI tickets i.e., Vaccinia virus, *Yersinia pestis*, *Francisella tularensis*, *Brucella melitensis*, and *Bacillus anthracis*, were declared valid for use in screening of the SIBCA exercise samples.

SIBCA exercise samples

Results of screening of SIBCA sample unknowns on MBI tickets are presented in Table 4. None of the SIBCA samples was found to contain Vaccinia virus, *Francisella tularensis*, or *Brucella melitensis*. Test sample #137 contained *Bacillus anthracis* and test sample #143 was positive for *Yersinia pestis*.

Identity of SIBCA samples

After all laboratories had completed their analyses and results had been reported, participant laboratories were notified by DPG of the contents of the SIBCA exercise samples sent to each nation. The identities of the five sample unknowns sent to Canada are presented in Table 5.

Agents identified by ICA in SIBCA samples

A summary of agents identified by ICAs (NHDC and/or MBI) is provided in Table 6. A comparison of agents identified by ICA with agents known present in SIBCA exercise samples is provided in Table 7. Results indicated that, in two of two samples for which ICAs were available and validated for use in the exercise, organisms were correctly identified i.e., 100% correct identification. Moreover, there were no false positive or false negative identifications.

Discussion

ICAs are installed as the first generation identification technology on the CIBADS. The SIBCA training exercise described herein provided an opportunity for DRES to evaluate "off-the-shelf" ICAs that were available commercially at the time of the training exercise. In addition, ICA analysis of SIBCA samples provided "back up," confirmatory capability for the Threshold® immunoassay system, the primary antibody-based identification technology employed during SIBCA exercise I [1, 3]. The ICAs evaluated herein reflect the commercial availability of ICAs for agents of military relevance at the time of SIBCA exercise I.

ICAs were purchased or gifted from one or both of two manufacturers i.e., NHDC and/or MBI. ICAs were available from one or both manufacturers for Vaccinia virus, *Yersinia pestis*, *Francisella tularensis*, *Brucella melitensis*, *Bacillus anthracis*, and *Vibrio cholerae*. ICAs were not available for *Burkholderia mallei*, VEE virus, Yellow fever virus, or *Coxiella burnetii*. Prior to the exercises, DRES had made informal arrangements with NHDC (David Trudil, NHDC, personal communication) to develop tickets for VEE and *Burkholderia mallei*. To this end, DRES sent *Burkholderia mallei* antibodies (two species) and antigen and VEE antibodies (two species) to NHDC for incorporation into ICA kits. However, no tickets were ever received from NHDC for use in identification of these two agents. At the time of writing, ICAs have become available, through the US JPO-BD for VEE and *Coxiella burnetii* and ICAs for *Burkholderia mallei* and Yellow fever virus are expected in the near future (David Cullin, US JPO- BD, personal communication).

ICAs from NHDC were received at DRES well in advance of the SIBCA training exercise. However, due to the small number of tickets received, it was not possible to evaluate them prior to the exercise and performance evaluation i.e., sensitivity and specificity had to be conducted during the exercises, in parallel with screening of the SIBCA sample unknowns. When tested for sensitivity and specificity, some NHDC tickets cross-reacted with other SIBCA agents included in the exercise. For example, *Bacillus anthracis* tickets cross-reacted with *Yersinia pestis*, *Francisella tularensis*, and *Brucella melitensis* antigens, and *Yersinia pestis* tickets cross-reacted with *Francisella tularensis* and *Brucella melitensis* antigens. Furthermore, some tickets reacted non-specifically, while others were non-reactive. For example, *Bacillus anthracis* tickets reacted non-specifically with PBS and water and *Brucella melitensis* tickets were non-reactive with all SIBCA antigens tested. Only *Vibrio cholerae* and *Francisella tularensis* tickets reacted with the homologous antigens and did not cross-react or react non-specifically with other agents and, therefore, these were the only two NHDC tickets validated for use in the SIBCA exercises. It is unclear why the NHDC tickets performed so poorly, but a contributing factor may have been that some of these tickets were provided by the manufacturer, and hence used in the training exercises, well beyond their expiry date. For example, *Yersinia pestis*, *Francisella tularensis*, and *Brucella melitensis* tickets had expired in June of the previous year. Only *Bacillus anthracis* and *Vibrio cholerae* tickets were not date expired prior to use in the exercise.

In addition to the above-noted problems, NHDC tickets were cumbersome to handle. Individual assays required multiple steps, including pre-mixing of component reagents prior to application of test sample. In addition, the system for delivery of test sample to sample well i.e., by drop rather than measured volume, was imprecise. On experimentation with the pipette droppers provided with the NHDC kits, it was found that the drop volume varied dependant upon location in the drop series and the pressure applied to the pipette bulb. In order to control for sample volume in the SIBCA training exercises, calibrated microliter pipettes were used rather than the disposable pipettes provided by NHDC. The multi-step format of the NHDC ICAs make these tickets unsuitable for use in military field scenarios where ticket handling is controlled by automation. Even in field situations that are operator controlled, the multiple step format requirement is an impediment to the soldier who has to function without the benefit of standard laboratory support and usually under the stress of threat of potential confrontation with biological attack.

The tickets manufactured by MBI and provided through the US JPO-BD were not received at DRES until the last designated day of the SIBCA training exercise. Therefore, there was insufficient time to evaluate these tickets for performance characteristics prior to commencement of the exercise. Furthermore, as with the NHDC tickets, there was a finite number of tickets available for use in the exercise and there were insufficient numbers to perform extensive tests and evaluation prior to the exercise. Thus, as with the NHDC tickets, the MBI tickets were tested for sensitivity and specificity in parallel with screening of the SIBCA sample unknowns. It was surprising to observe that the MBI tickets for *Yersinia pestis*, *Brucella melitensis*, and *Bacillus anthracis* did not react with the respective homologous inactivated antigens at any of the wide range of concentrations tested. Similarly, there was no cross-reactivity with any of the inactivated heterologous antigens. Only the *Francisella tularensis* ticket reacted specifically with homologous inactivated *Francisella tularensis* antigen. In light of these observations, the manufacturer, MBI, was contacted and their advice sought on how these problems might be resolved. MBI personnel responded that their tickets had been manufactured with antibodies designed to detect live agent i.e., the immunogens used to develop the antibodies incorporated in the tickets had been either live or gamma-irradiated, and MBI proposed that this might be the reason for non-reactivity of their tickets with the formalin-inactivated material used by DRES as positive agent controls. As a last resort in demonstrating the validity of the MBI ICAs, tickets for *Yersinia pestis*, *Brucella melitensis*, and *Bacillus anthracis* were taken into the DRES biocontainment level 3 laboratories and challenged with unspecified amounts of live homologous agents. All tickets tested gave strong positive signals with the homologous live agent. As the last remaining *Yersinia pestis*, *Brucella melitensis*, and *Bacillus anthracis* tickets had been used to demonstrate reactivity with the live agent, there were no tickets remaining to conduct specificity studies with live heterologous agents. Similarly, for the same reasons, Vaccinia virus and *Francisella tularensis* tickets were not tested for specificity against live heterologous agents. It is interesting to note that Vaccinia virus tickets reacted well with Vaccinia virus antigen, which is a live virus vaccine. None of the MBI tickets reacted non-specifically with PBS or water. In spite of the fact that MBI tickets were not assessed at DRES for cross-reactivity, MBI personnel confirmed that tickets had undergone extensive quality control testing at the manufacturing level and had been shown to be agent-specific. Based on these assurances, it was decided to validate the MBI tickets for use in the SIBCA exercises.

With respect to ease of handling and precision, the MBI tickets were superior to the NHDC tickets. MBI ICAs are conducted in a single step, by addition of a measured volume of sample to the sample well, and results are read after a 10 min reaction period. The single-step format of the MBI tickets is amenable to either manual or automated operations in a military field scenario. In addition, because of the simplicity and ease with which assays are performed, these tickets are suitable for use by soldiers operating under the stress of threat of biological attack.

Four of the five SIBCA samples provided by DPG contained biological agent and one of the five samples consisted of buffer only (Table 5). DRES correctly identified those agents for which ICAs were available and had been validated. Thus, *Bacillus anthracis* and *Yersinia pestis* were correctly identified in samples #147 and #143, respectively, representing a positive identification rate of 2/2 = 100%. There were no false positive identifications (0% false positive rate) i.e., no agent was identified in sample #110, containing buffer only, or in samples #160 or #157, containing *Coxiella burnetii* and VEE, respectively, neither of which could be tested for by ICAs available to DRES at the time of the training exercise. Similarly, there was 100% correlation between the results of SIBCA exercise I samples analysed by ICA and by the Threshold^{R} immunoassay [1].

Examination of ICA results suggests that there were differences in assay reactivity of homologous antigen that were related to the nature of the antigen used to induce the detecting antibodies i.e., whether live or inactivated and, if inactivated, the method of inactivation, and the nature of the antigens assayed i.e., whether live, formalin-inactivated, or cobalt-inactivated. For example, in the anthrax ICA, which incorporated detecting antibodies raised against cobalt-irradiated agent, the anthrax positive control antigen (which was formalin-inactivated) was negative, while live agent and cobalt-irradiated agents were positive. These observations are consistent with an interpretation that anthrax present in the formalin-inactivated positive control material had undergone changes to the extent that altered epitopes on the agent surface were undetectable by antibodies raised against pristine (i.e., live or cobalt-irradiated) anthrax antigen. That inactivation of proteins with formalin can result in damage to surface epitopes, is well documented. Kabat and Myers [4] reported that extensive, irreversible cross-linking of amino acid side chains can occur under conditions of formaldehyde inactivation used to produce toxoids and vaccines. Furthermore, proteins reacted with formaldehyde have been shown to acquire added antigenic specificity and antisera prepared against these altered proteins usually cross-react with other formaldehyde-treated proteins [5]. Perhaps the most celebrated example of epitope alteration believed due to the effects of formaldehyde is the formalin-inactivated measles vaccine that was used in the US from 1963-67. This vaccine failed to induce in recipients protective antibodies against one of the virus epitopes, with the result that only transient protection against virulent virus was achieved [6]. The anthrax positive agent control used in the SIBCA training exercise was inactivated at DRES with 3.7% formalin (John Cherwonogrodzky, personal communication). Evidence suggestive that this material may have been damaged comes from anecdotal reports that the preparation exhibited properties of severe clumping, consistent with cross-linking of component proteins. Gamma irradiation of organisms, as a means of inactivation of infectivity, acts by fragmentation of component nucleic acids. This method of inactivation, because it acts on nucleic acid rather than surface protein, is believed not to be damaging to the integrity of surface protein epitopes and, therefore, is the favoured method of inactivation for retention of antigenicity. As observed in the *Bacillus anthracis* ICA, ICAs for *Yersinia*

pestis and *Brucella melitensis*, both of which incorporated detecting antibodies raised against cobalt-irradiated agents, were negative when tested with formalin-inactivated positive agent controls; whereas, when these same tickets were tested with live agent and cobalt-irradiated agents, they were positive. These observations support the hypothesis that assay performance is influenced by the method of inactivation of the antigen used to produce the detecting antibodies incorporated in the assay. Whether antibodies produced from formalin-inactivated agents could detect live agent was not assessed and is unknown. Further studies are required to determine the effects of various methods of inactivation on the antigenicity of specific biological agents and subsequent effects on immunoreactivity and biodetection capability of antibodies raised in response to these antigens. Agent-specific research in this area is currently underway both at US Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD (Peter Emmanuel, personal communication) and at US DPG (Bruce Harper, personal communication). Until the results of these important studies are known, it would be advisable that methods used for inactivation of agents for use as immunogens in the production of biodetection antibodies, be those least likely to result in damage to surface protein epitopes. Present consensus in this regard is that gamma irradiation e.g., cobalt irradiation, is the best procedure for inactivating the infectivity of microorganisms while, at the same time, retaining their molecular integrity and antigenicity.

Conclusions

Sample unknowns from SIBCA exercise I were screened by ICAs purchased or gifted from two commercial sources. Concurrent to screening of SIBCA sample unknowns by ICA, ICAs were evaluated, for sensitivity in detection of the homologous agents, and specificity against a panel of heterologous agents. Comparison of results obtained by ICA screening of SIBCA sample unknowns, with the known content of agents in SIBCA samples, indicated that, for agents for which ICAs were available and validated for use in the training exercise, there was 100% correct identification of agents in samples, thus demonstrating the potential utility of ICAs as immunodiagnostic tools for identification of biological agents of military relevance. The results described in this report reflect the state-of-the-art in development of ICAs for agents of concern to the CF at the time of SIBCA training exercise I. Problems relating to ICA availability, ICA ease of handling, assay cross-reactivity, and assay non-reactivity were encountered and are described in the report. An important observation made during the course of this work was that ICAs that incorporated antibodies that had been developed in response to gamma-irradiated agents, could detect gamma-irradiated and live agents, but could not detect formalin-inactivated agents. The implications of this finding, including the proposal that assay performance is influenced by the method of inactivation of agent used to induce the biodetecting antibody, are discussed in the report.

Table 1. Sensitivity and specificity of NHDC tickets for detection and identification of homologous and heterologous agents

Test agent	Ticket[1]					
	V. cholerae	*Y. pestis*	*F. tularensis*	*B. melitensis*	*B. anthracis*	
					Lot 504	Lot 503A
V. cholerae.						
(kit standard)	+	nd	nd	nd	nd	nd
Y. pestis (cfu/mL):						
1X10E6	nd	+*	nd	nd	nd	nd
1X10E7	nd	+*	nd	nd	nd	nd
1X10E8	-	-	-	-	+	nd
F. tularensis (cfu/mL)						
1.1X10E7	nd	nd	+	nd	nd	nd
1.1X10E8	-	+/-	-	-	+	+*
B. melitensis (cfu/mL):						
1 8X10E5	nd	nd	nd	-	nd	nd
1 8X10E7	nd	nd	nd	-	nd	nd
1 8X10E8	-	+/-	-	-	+	+*
B. anthracis (cfu/mL):						
2.2X10E7	-	-	-	-	+	+*
Smart™ wash solution	-	-	-	-	nd	nd
PBS	-	-	-	-	+*	nd
NANOpure® water	-	-	-	-	+*	nd

+: positive; -: negative; nd: not done

+/-: one of duplicate tickets negative

*: result from single determination

[1] Unless otherwise indicated, results are from duplicate determinations.

Table 2. SIBCA sample unknowns tested by NHDC tickets

Sample no.	Ticket[2]					
	V cholerae Lot 136	*Y. pestis* Lot 302	*F. tularensis* Lot 800	*Brucella* Lot 900	*B. anthracis*	
					Lot 504	Lot 503A
110	-	+/-	-	-	+	-*
137	-	-	-	-	+	+*
143	-	+	-	-	+	+*
157	-	+/-	-	-	+	-*
160	-	-	-	-	+	+*

+. positive, -: negative

+/-: one of duplicate tickets negative

*: result from single determination

[2] Unless otherwise indicated, results are from duplicate determinations.

Table 3. Sensitivity and specificity of MBI tickets for detection and identification of homologous and heterologous agents

Test agent	Ticket[3]				
	Vaccinia virus	*Y. pestis*	*F. tularensis*	*B. melitensis*	*B. anthracis*
Vaccinia virus (doses/mL):					
0.05	-	-	-	-	-
5.0	+*	nd	nd	nd	nd
Y. pestis, killed (cfu/mL):					
1X10E6	nd	-*	nd	nd	nd
1X10E7	-	nd	-	-	-
1X10E9	nd	nd	nd	nd	nd
1X10E9 boiled[4]	nd	-*	nd	nd	nd
1X10E10	nd	-*	nd	nd	nd
Y. pestis, live	nd	+*	nd	nd	nd
F. tularensis, killed (cfu/mL).					
1.1X10E7	-	-	+	-	-
B. melitensis, killed (cfu/mL):					
1.8X10E6	nd	nd	nd	-*	nd
1.8X10E7	-	-	-	-	-
1.8X10E9	nd	nd	nd	-	nd
1.8X10E10	nd	nd	nd	-*	nd
B. melitensis, live	nd	nd	nd	+*	nd
B. anthracis, killed (cfu/mL)					
2.2X10E5	nd	nd	nd	nd	-*
2.2X10E6	-	-	-	-	-
2.2X10E8	-	-	-	-	-
1.1X10E9	nd	nd	nd	nd	-*
2.2X10E9	nd	nd	nd	nd	-*
NHDC+ve control[5]	nd	nd	nd	nd	-*
B. anthracis, live	nd	nd	nd	nd	+*
PBS	-*	-*	-*	-*	-*
NANOpure® water	-*	-*	-*	-*	-*

+: positive, -: negative; +/-: one of duplicate tickets negative

*: result from single determination

[3] Unless otherwise indicated, results are from duplicate determinations.
[4] *Y. pestis* boiled, ~1 mg/mL (SciLab Inc., Medicine Hat, AB)
[5] *B. anthracis* Smart™ kit: positive control, Lot 100, Exp. 220891 (NHDC)

Table 4. SIBCA sample unknowns tested by MBI tickets

Sample no.	Ticket[6]				
	Vaccinia	*Y. pestis*	*F. tularensis*	*B. melitensis*	*B. anthracis*
110	-	-	-	-	-
137	-	-	-	-	+
143	-	+	-	-	-
157	-	-	-	-	-
160	-	-	-	-	-

+: positive; -. negative,

Table 5. Known contents of SIBCA exercise I samples

Sample no.	Agent	Concentration
110	*PBS*	
137	*Bacillus anthracis*, vollum strain	8 7X10E6 clu/mL; 6.1 µg/mL (protein)
143	*Yersinia pestis*, Lapaz strain	3 7X10E7 clu/mL
157	VEE virus, vaccine strain TC83	7.5X10E8 TCID 50/mL; 0 5 µg/mL[7] (protein)
160	*Coxiella burnetii*, 9 mile strain, phase I	2.1X10E7 ID_{50}/mL; 2.3 µg/mL (optical density)

[6] Results are from duplicate determinations.
[7] 7.5X10E7 cfu/mL actually provided

Table 6. Summary of agents identified by ICA in SIBCA exercise I samples

Sample no.	Ticket					
	Vaccinia[a]	*Y. pestis*[a]	*F. tularensis*[a,b]	*B. melitnsis*[a]	*B. anthracis*[a]	*V. cholerae*[b]
110	-	-	-	-	-	-
137	-	-	-	-	+	-
143	-	+	-	-	-	-
157	-	-	-	-	-	-
160	-	-	-	-	-	-

[a]: MBI; [b]: NHDC

+: positive; -: negative;

Table 7. Agents identified by ICA compared to agents known present in SIBCA exercise I samples

Sample no.	Agent present	Agent identified
110	PBS	none
137	*B. anthracis*	*B. anthracis*
143	*Y. pestis*	*Y. pestis*
157	VEE	na[8]
160	*C. burnetii*	na[9]

[8] VEE tickets not available (na)

[9] *C. burnetii* tickets not available (na)

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FULTON, R. ELAINE and STADNYK, LAURIE L.

5 DATE OF PUBLICATION (month and year of publication of document)	6a. NO. OF PAGES (total containing information, include Annexes, Appendices, etc)	6b. NO OF REFS (total cited in document)
September 2000	32	6

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In March 1999, the NATO Panel VII Subgroup on Sampling and Identification of Biological and Chemical Agents (SIBCA) conducted the first international training exercise on identification of biological agents. Eleven NATO national laboratories participated in the exercise: Canada, France, Germany, (two laboratories), Hungary, Italy, Netherlands, Norway, Poland, United Kingdom, and United States. Participating laboratories were sent five sample unknowns, four of which contained biological agents, and one blank containing buffer only. Participants were advised that biological agents could consist of any one of the following 10 gamma-irradiated organisms: *Bacillus anthracis*, *Yersinia pestis*, *Vibrio cholerae*, Venezuelan Equine Encephalitis (VEE) virus, *Francisella tularensis*, *Brucella melitensis*, *Burkholderia mallei*, Yellow Fever virus, Vaccinia virus, or *Coxiella burnetii*. The participating laboratory for Canada was the Defence Research Establishment Suffield (DRES). For the training exercise, DRES screened sample unknowns by two different antibody-based identification technologies, one of which was the immunochromatographic assay (ICA). ICAs for six of the 10 possible biological agents were available from one or both of two commercial sources and SIBCA samples were screened for these six agents. Cross-reactivity of some ICAs with heterologous agents, as well as non-reactivity of some positive agent control materials on some ICAs, were observed. However, comparison of ICA results with the known identity of agents in SIBCA sample unknowns indicated 100% correct identification of agents for which ICAs were available and validated.

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Biological agents
Bacillus anthracis
Yersinia pestis
Vibrio cholerae
Venezuelan Equine Encephalitis (VEE) virus
Francisella tularensis
Brucella melitensis
Burkholderia mallei
Yellow Fever virus
Vaccinia virus
Coxiella burnetii
Identification
Training exercise
Immunochromatographic assay (ICA)
Antibody-based hand-held test kit (HHTK)
Antibody-based hand-held assay (HHA)
Ticket
Lateral flow device